



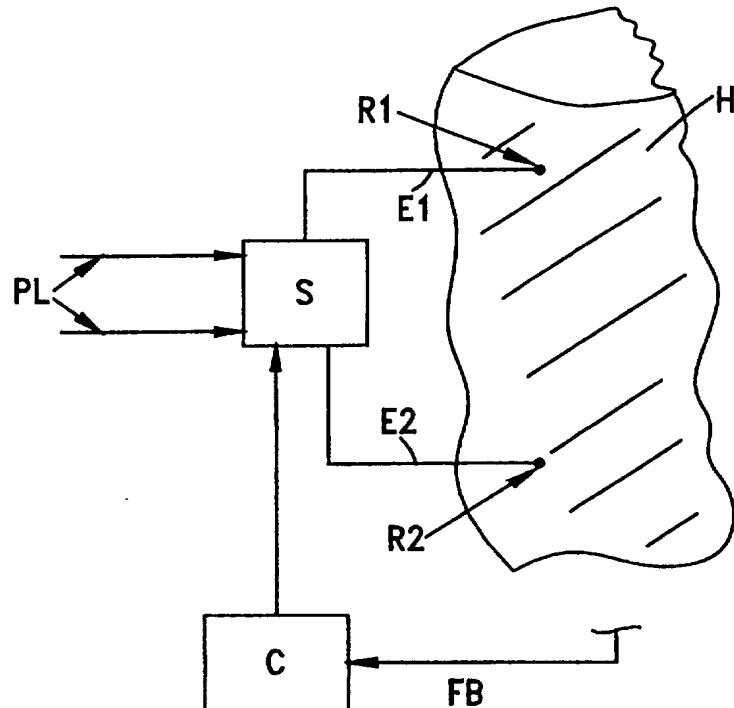
## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>6</sup> :  A61N 1/362		A1	(11) International Publication Number: <b>WO 98/10829</b>
(21) International Application Number: PCT/IL97/00232		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).	(43) International Publication Date: 19 March 1998 (19.03.98)
(22) International Filing Date: 9 July 1997 (09.07.97)			
(30) Priority Data:			
60/026,392 119261	16 September 1996 (16.09.96) 17 September 1996 (17.09.96)	US IL	
(71) Applicant (for all designated States except US): NEW TECHNOLOGIES (SA-YSY) LTD. [IL/IL]; P.O. Box 8044, 31080 Haifa (IL).			Published With international search report.
(72) Inventors; and			
(75) Inventors/Applicants (for US only): BEN-HAIM, Shlomo [IL/IL]; Yeffy Nof Avenue 101, 34454 Haifa (IL). DARVISH, Nissim [IL/IL]; Hantke Street 22a, 34606 Haifa (IL). MIKA, Yuval [IL/IL]; Bet-Lechem Street 49, 35567 Haifa (IL). FENSTER, Maier [IL/IL]; Brande Street 61, 49600 Petach Tikva (IL).			
(74) Agents: LUZZATTO, Kfir et al.; Luzzatto & Luzzatto, P.O. Box 5352, 84152 Beer-Sheva (IL).			

## (54) Title: DRUG-DEVICE COMBINATION FOR CONTROLLING THE CONTRACTILITY OF MUSCLES

## (57) Abstract

An apparatus for the combined drug/electric-stimulation treatment of a cardiac muscle comprises circuitry (S) for creating a non-excitatory electric potential between at least two points (R1, R2) located in the vicinity of a muscle (H). A method for the combined drug/electric-stimulation treatment of a cardiac muscle, using the apparatus, involves controlling the start time, duration, and magnitude of the electric current flowing between said at least two points, to impart a desired change in cardiac muscle performance.



**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

<b>AL</b>	Albania	<b>ES</b>	Spain	<b>LS</b>	Lesotho	<b>SI</b>	Slovenia
<b>AM</b>	Armenia	<b>FI</b>	Finland	<b>LT</b>	Lithuania	<b>SK</b>	Slovakia
<b>AT</b>	Austria	<b>FR</b>	France	<b>LU</b>	Luxembourg	<b>SN</b>	Senegal
<b>AU</b>	Australia	<b>GA</b>	Gabon	<b>LV</b>	Latvia	<b>SZ</b>	Swaziland
<b>AZ</b>	Azerbaijan	<b>GB</b>	United Kingdom	<b>MC</b>	Monaco	<b>TD</b>	Chad
<b>BA</b>	Bosnia and Herzegovina	<b>GE</b>	Georgia	<b>MD</b>	Republic of Moldova	<b>TG</b>	Togo
<b>BB</b>	Barbados	<b>GH</b>	Ghana	<b>MG</b>	Madagascar	<b>TJ</b>	Tajikistan
<b>BE</b>	Belgium	<b>GN</b>	Guinea	<b>MK</b>	The former Yugoslav Republic of Macedonia	<b>TM</b>	Turkmenistan
<b>BF</b>	Burkina Faso	<b>GR</b>	Greece	<b>ML</b>	Mali	<b>TR</b>	Turkey
<b>BG</b>	Bulgaria	<b>HU</b>	Hungary	<b>MN</b>	Mongolia	<b>TT</b>	Trinidad and Tobago
<b>BJ</b>	Benin	<b>IE</b>	Ireland	<b>MR</b>	Mauritania	<b>UA</b>	Ukraine
<b>BR</b>	Brazil	<b>IL</b>	Israel	<b>MW</b>	Malawi	<b>UG</b>	Uganda
<b>BY</b>	Belarus	<b>IS</b>	Iceland	<b>MX</b>	Mexico	<b>US</b>	United States of America
<b>CA</b>	Canada	<b>IT</b>	Italy	<b>NE</b>	Niger	<b>UZ</b>	Uzbekistan
<b>CF</b>	Central African Republic	<b>JP</b>	Japan	<b>NL</b>	Netherlands	<b>VN</b>	Viet Nam
<b>CG</b>	Congo	<b>KE</b>	Kenya	<b>NO</b>	Norway	<b>YU</b>	Yugoslavia
<b>CH</b>	Switzerland	<b>KG</b>	Kyrgyzstan	<b>NZ</b>	New Zealand	<b>ZW</b>	Zimbabwe
<b>CI</b>	Côte d'Ivoire	<b>KP</b>	Democratic People's Republic of Korea	<b>PL</b>	Poland		
<b>CM</b>	Cameroon			<b>PT</b>	Portugal		
<b>CN</b>	China	<b>KR</b>	Republic of Korea	<b>RO</b>	Romania		
<b>CU</b>	Cuba	<b>KZ</b>	Kazakhstan	<b>RU</b>	Russian Federation		
<b>CZ</b>	Czech Republic	<b>LC</b>	Saint Lucia	<b>SD</b>	Sudan		
<b>DE</b>	Germany	<b>LI</b>	Liechtenstein	<b>SE</b>	Sweden		
<b>DK</b>	Denmark	<b>LK</b>	Sri Lanka	<b>SG</b>	Singapore		
<b>EE</b>	Estonia	<b>LR</b>	Liberia				

**DRUG-DEVICE COMBINATION FOR CONTROLLING THE**

**CONTRACTILITY OF MUSCLES**

**Field of the Invention**

The present invention relates to the field of medicine. More particularly, the invention relates to means for controlling the contractility of muscles in patients treated with drugs which influence the cardiovascular system.

**Background of the Invention**

Many activities of the human body involve the contraction of muscles. For instance, movement of the limbs, breathing activity, etc. The most complex and vital muscular activity of the human body is that of the heart, which functions as a pump and which, by contracting at the required times and in the required manner, controls the flow of blood throughout the body.

The heart is composed of different parts, which contract differently and with different timing, in order to permit the aforementioned pumping activity. The contraction of the heart is controlled by electric stimuli, which are generated at the cellular level by chemical reaction. However, it is well known in the art to control the timing of the contraction of the cardiac muscle, by the action of externally applied electric stimuli, through the so-called "pace maker".

In a copending PCT patent application No. PCT/IL97/00012, filed January 8, 1997 by the same applicants herein, the specification of which is incorporated herein by reference, there is described a method and apparatus for modifying the force contraction of at least a portion of a heart chamber, which comprises applying a non-excitatory electric field, for a predetermined period of time, at a delay after activation, which causes the force of contraction to be increased. Substantial increases in the force of contraction are obtained, typically - but non-limitatively - in the order of 5% - 50%. This increase in cardiac output is useful in order to obviate cardiac insufficiency due to a variety of pathological situations, e.g., the reduction of cardiac output due to the implantation of a pace maker, the insufficiency due to the results of the malfunctioning of a portion of the cardiac muscle, etc.

In another copending PCT/IL patent application, entitled "Apparatus and Method for Controlling the Contractility of Muscles", filed by the same applicants herein on the same day as the present application and identified as Attorney's Docket 4224/WO/97, the specification of which is incorporated herein by reference, there is described a method and apparatus for decreasing the force contraction of at least a portion of a heart chamber, which comprises applying a non-excitatory electric field of a polarity inverse of that required for obtaining an increase of muscle contractility, for a predetermined period of time, at a delay after

activation, which causes the force of contraction to be decreased. The ability of reducing the contractility is of importance in a variety of situations, e.g., during surgery or as an aid in healing of hibernated areas of a heart after myocardial infarct.

Several severe diseases of the cardiac muscle cannot be treated effectively without the aid of drugs. However, many drugs used in therapy have a deleterious side-effect in that they reduce the contractility and cardiac output of the heart and may cause life threatening arrhythmias. Some illustrative examples of drugs used in the treatment of congestive heart failure (CHF) are given below.

Congestive heart failure (CHF) is a complex clinical syndrome characterized by impaired ventricular performance, exercise intolerance, a high incidence of ventricular arrhythmias, and shortened life expectancy. Virtually all forms of heart disease can lead to heart failure, with coronary artery disease, hypertension, and diabetes mellitus being the most common in the U.S.

CHF can be broadly subdivided into two distinct forms (although several other classification schemes have been used). Distinguishing between the two forms is often difficult: 1. Diastolic dysfunction or diastolic heart failure; and 2. Systolic dysfunction or systolic CHF.

Changes in the structure and biochemical properties of the myocardium and peripheral vasculature occur during the development of CHF and these changes can contribute to further impairment of cardiovascular function. Many different drugs are used in the therapy of CHF, which affect the cardiac muscle, whether directly or indirectly, by causing changes in the cardiovascular system.

Recent studies indicate that metoprolol, a commonly used beta-blocker which can cause up-regulation of beta-1 receptors, can restore catecholamine responsiveness to patients with dilated cardiomyopathy.

Captopril, an ACE inhibitor that is commonly used to treat heart failure, has been shown to increase lymphocyte Gs and increase myocardial beta-1 receptor density.

Digitalis (cardiac glycosides): has been used clinically for over 200 years to treat heart failure and edema (dropsy), but its present use in treating CHF is controversial. Digoxin is the most widely used preparation of digitalis (half-life = 1-2 days), although digitoxin (half-life = 7 days) is also used in situations where long half-life may be an advantage.

However, Digoxin may be toxic at relatively low dosages, since it may cause arrhythmia and conduction problems, but the art has so far failed to

provide means by which the desirable therapeutic effect of Digoxin or similar drugs may be maintained at low dosages, so as to avoid toxicity problems.

Beta-Adrenergic agonists: beta1-adrenergic agonists (dopamine, dobutamine, prenalterol, xamoterol) have been used to treat acute and chronic heart failure, but have limited usefulness in chronic CHF because of their arrhythmogenic effects, short duration of action, the development of tolerance, and necessity of parenteral administration.

Dobutamine is a moderately selective beta1-adrenergic agonist that lacks vasoconstrictor activity and causes minimal changes in heart rate. It is frequently added to nitroprusside when blood pressure is adequate to increase cardiac output.

Prenalterol and xamoterol are partial beta1-adrenergic agonists that may simultaneously stimulate beta1-receptors and block the receptors from stimulation by endogenous catecholamines, thereby protecting against beta1-receptor down-regulation.

Cyclic nucleotide phosphodiesterase (PDE-III, cGMP-inhibitable PDE)

inhibitors: There are several agents that increase myocardial and vascular smooth muscle cAMP through inhibition of cyclic nucleotide

phosphodiesterase PDE-III, cGI PDE) activity. These agents should therefore simultaneously increase cardiac output and reduce afterload.

The bipyridines, amrinone and milrinone, are potent PDE-III inhibitors that can be given orally or parenterally. They can be given orally, are generally well-tolerated, but can have significant non-cardiac side-effects (nausea, vomiting, thrombocytopenia).

Ca<sup>+</sup> Channel Blockers: Ca<sup>+</sup> channel antagonists are widely used in many types of heart diseases, including cardiac insufficiency, and cause direct arterial vasodilatation by the inhibition of Ca<sup>+</sup> current. Diltiazem and Verapamil exhibit negative inotropic, chronotropic and dromotropic effect on cardiac function. Some of these drugs, such as Verapamil, have life threatening side effects, such as altering the A-V node conduction.

Anti-Arrhythmic Drugs: In cardiac insufficiency, and in other ischemic diseases, many patients suffer from arrhythmia. Drugs acting on the chronicity are known to cause many side effects.

It is therefore clear that it is highly desirable to be able to reduce the dosage of such drugs, whenever possible, or to obviate side effects and problems which they may cause.

It has now been surprisingly found, and this is an object of the present invention, that it is possible to obviate the aforementioned drawbacks of drugs used in the therapy of heart diseases, by applying a non-excitatory signal of the type described above, which increases the contractility of the cardiac muscle and its output. It has further been found that this can be achieved without generating adverse effects and/or without adversely affecting the activity of the drug which is administered to the patient.

It is an object of the invention to provide apparatus for the combined drug/electric-stimulation treatment, which can be used as a treatment aid for a patient in need of a medicine which impairs heart muscle contractility.

It is another object of the invention to provide a method for treating a patient in need of a drug which may impair the contractility of the heart muscle.

It is yet another object of the invention to provide apparatus useful in therapy, by means of which the dosage of drugs having potentially harmful side effects can be reduced or, alternatively, by means of which the dosage can be increased while keeping side effects within an acceptable range.

It is still another object of the invention to provide apparatus which cooperates with such drugs, its activity being related to the amount of the drug present at a given time in the patient's blood.

Other objects and advantages of the invention will become apparent as the description proceeds.

#### SUMMARY OF THE INVENTION

In one aspect, the invention relates to an apparatus for the combined drug/electric-stimulation treatment of a cardiac muscle, comprising circuitry for creating a non-excitatory electric potential between at least two points located in the vicinity of a muscle.

According to a preferred embodiment of the invention, the apparatus comprises circuitry for controlling the start time of the electric potential generated between said at least two points. According to another preferred embodiment of the invention the apparatus comprises circuitry for controlling the duration of the electric potential generated between said at least two points. According to still another preferred embodiment of the invention the apparatus comprises circuitry for controlling the magnitude of the electric potential generated between said at least two points.

In a preferred embodiment of the invention the circuitry for creating a non-excitatory electric potential between said at least two points comprises one or more electrode. A variety of different electrodes can be used, which will be easily recognized by the skilled person. An illustrative example of suitable electrodes are carbon electrodes.

In another aspect, the invention is directed to apparatus for the combined drug/electric stimulation treatment, comprising circuitry for causing a non-excitatory electric current to flow between at least two points located in the vicinity of a muscle.

According to a preferred embodiment of the invention, the apparatus comprises circuitry for controlling the start time of the electric current flowing between said at least two points.

According to another preferred embodiment of the invention, the apparatus comprises circuitry for controlling the duration of the electric current flowing between said at least two points.

According to still another preferred embodiment of the invention, the apparatus comprises circuitry for controlling the magnitude of the electric current flowing between said at least two points.

In a preferred embodiment of the invention the circuitry for causing a non-excitatory electric current to flow between said at least two points comprises one or more electrode, e.g., carbon electrodes.

In another aspect the invention is directed to means and methods for counteracting proarrhythmic effects of drugs.

In the context of the present invention, by "non-excitatory current", or "non-excitatory potential", or "non-excitatory signal", it is meant to indicate a signal which does not cause a propagating action potential in the muscle cells (which may start a new pacing or contraction of the muscle). In other words, the non-excitatory electric stimulation effected by a non-excitatory electric pulse is such that it does not induce propagating activation potentials in the cardiac muscle cells. Rather, such pulses affect the response of the heart muscle to the action potentials, by modulating cell contractility within selected segments of the cardiac muscle. As described in the abovementioned PCT patent application PCT/IL97/00012, the inventors have found that by applying non-excitatory electrical stimulation pulses of suitable strength, appropriately timed with respect to the heart's electrical activation, the contraction of the selected segments can be increased or decreased, thus increasing or decreasing the stroke volume of the heart.

There may be various reasons for a signal to be non-excitatory. Two main types of non-excitatory signals to be used in conjunction with the invention are: 1) A signal which, independently of its magnitude, is applied during the refractory period, and therefore does not cause a new contraction, even though its magnitude may be above threshold values for pacing; 2) A signal which is sub-threshold for pacing and, therefore, no matter when given, does not cause a new contraction to take place.

While a DC current is typically used as the base line for the non-excitatory signal, it is possible to supply a signal which is a complex signal, for instance, a signal generated by superimposing an AC current on the DC base signal, so as to generate a waveform of varying envelope. Any suitable signal can be superimposed, having any shape, e.g., square wave or sinusoidal wave, as will be apparent to the skilled person. Thus, according to one preferred embodiment of the invention the apparatus further comprises means for superimposing on a DC signal one or more waveforms of given frequency and amplitude, thereby to generate a complex signal.

The apparatus of the invention can be provided in different forms, e.g., as an insertable device or an extra corporal device or an implantable device.

According to a preferred embodiment of the invention, the circuitry for controlling the start time and/or duration of the electric potential is

synchronized to heart activity, for instance, it can operate not at every beat of the heart, e.g., every 1, 2 or 3 beats of the heart.

According to a preferred embodiment of the invention the non-excitatory electric current is a DC electric current, and the apparatus may further comprise circuitry for superimposing on the DC signal one or more waveforms of given frequency and amplitude, thereby to generate a complex signal.

The apparatus employed to carry out the method of the invention can be of different construction, as will be apparent to the skilled person. One example of apparatus suitable for carrying out the invention is described in detail and claimed in a copending PCT patent application of the same applicants herein, entitled "Cardiac Output Controller", filed on the same day as the present application and identified as Attorney's Docket 27068, the description of which is incorporated herein by reference. Another example of suitable apparatus, coupled to a pacemaker device, is the subject of another copending PCT patent application of the same applicants herein, entitled "Cardiac Output Enhanced Pacemaker", filed on the same day as the present application and identified as Attorney's Docket 27181, the specification of which is also incorporated herein by reference. However, as said, the invention is not intended to be limited to any particular construction of device used to carry it out.

In another aspect, the invention is directed to apparatus for the combined drug/electric-stimulation treatment of a cardiac muscle, comprising:

- means for creating an electric potential between at least two points located in the vicinity of the cardiac muscle;
- means for causing a non-excitatory electric current to flow between said at least two point; and
- means for controlling the start time, duration and magnitude of the electric current flowing between said at least two points.

According to a preferred embodiment of the invention the apparatus comprises:

- means for creating an electric potential between at least a pair of electrodes in the vicinity of the cardiac muscle at at least two root locations;
- means for causing a non-excitatory electric current to flow between said at least two root locations; and
- means for controlling the start time, duration and magnitude of the electric current flowing between said at least two root locations.

By "root location" it is meant to indicate the vicinity of the muscle where the electrodes are located, which may be distinct from the area which is affected by the current flowing between them. As will be appreciated by the skilled person, due to the very complex nature of the electric behavior

of the cardiac muscle, it is possible that positioning an electrode at a given location will affect another, more remote portion of the muscle. Therefore, the root location is not necessarily the center or any other portion of the treated area, but it is only a location, near the muscle, where an electrode will be positioned.

The invention is further directed to a method for the combined drug/electric stimulation treatment of a cardiac muscle, comprising a) administering to a patient in need thereof a drug which affects the cardiovascular system and which affects the cardiac muscle, or a mixture of two or more such drugs, b) creating a non-excitatory electric potential between at least two points located in the vicinity of the muscle, and c) controlling one or more of the parameters consisting of start time, duration, magnitude and polarity of the non-excitatory electric potential created between said at least two points.

In another aspect the invention is directed to a method for the combined drug/electric stimulation treatment of a cardiac muscle, comprising a) administering to a patient in need thereof a drug which affects the cardiovascular system and which affects the cardiac muscle, or a mixture of two or more such drugs, b) causing a non-excitatory electric current to flow between at least two points located in the vicinity of the muscle, and c) controlling one or more of the parameters consisting of start time,

duration, magnitude and polarity of the non-excitatory electric current flowing between said at least two points.

The signal supplied to the patient according to the method of the invention is of the same type as described above with reference to the apparatus.

The change in cardiac muscle contractility and of electrophysiological characteristics of the cardiac muscle obtained by the method of the invention can be exploited for a variety of therapeutic purposes. According to one preferred embodiment of the invention, it is designed at least partially to compensate for cardiac muscle contractility decrease induced by said drug or mixture of drugs. According to another preferred embodiment of the invention it is designed at least partially to compensate for cardiac muscle contractility increase induced by said drug or mixture of drugs. According to still another preferred embodiment of the invention the change in cardiac muscle contractility is designed to add to and/or amplify the cardiac muscle contractility increase or decrease induced by said drug or mixture of drugs.

In another aspect the invention is directed to a method for reducing the dosage of a cardiovascular drug which affects cardiac muscle contractility. Thus, drugs such as Digoxin, and other proarrhythmic drugs, which as explained above have serious side effects, can be administered in

substantially lower dosages, while at the same time achieving the same or even an improved effect on cardiac muscle contractility.

Thus, according to a preferred embodiment of the invention there is provided a method for the combined drug/electric-stimulation treatment of a cardiac muscle, comprising:

- administering to a patient in need thereof a drug which affects the cardiovascular system and which affects the cardiac muscle, or a mixture of two or more such drugs;
- providing an electric potential between at least a pair of electrodes in the vicinity of the cardiac muscle at at least two root locations;
- causing a non-excitatory electric current to flow between said at least two contacting locations; and
- controlling the start time, duration and magnitude of the electric current flowing between said at least two root locations, so as to impart the desired change in cardiac muscle contractility.

The invention further encompasses a kit for the treatment of cardiovascular diseases, comprising a drug or a combination of two or more drugs, and an apparatus according to the invention, as herein described.

Additional apparatus according to the invention comprises circuitry for controlling its activity as a function of calculated and/or actual level of a given drug or combination of drugs in the patient's blood.

The apparatus of the invention is further useful for reducing the dosage of pro-arrhythmic drugs, and, for counteracting side-effects of pro-arrhythmic drugs.

Further therapeutic flexibility can be provided by another preferred embodiment of the invention, in which the apparatus comprises timing circuitry for activating it at predetermined times during the day.

As will be appreciated by the skilled person, the actual set of operating parameters used (current, length of pulse, number of electrodes, lag after pacing signal, etc.), will be dependent on the specific use made of the invention, and the skilled person will be able to devise the optimal set of parameters for a given application. Some non-limitative operating ranges in which cardiac muscle reacts according to the invention are given here for the sake of illustration only, it being understood that operation outside such ranges is of course possible under various conditions: Current: 0.01 - 10 mA; Length of Pulse: 1-998 milliseconds with a pacing of 1 Hz, and 1-498 milliseconds with a pacing of 2 Hz; Delay after Pacing Signal: 1 milliseconds and above. Where no pace maker is used, the delay is

preferably calculated from the natural pacing of the patient's heart, or from the local activation time.

While a variety of electrodes can be used, and the invention is in no way limited to any particular type of electrode, particularly preferred suitable electrodes for this purpose are, e.g., carbon electrodes.

#### **Brief Description of the Drawings**

The above and other characteristics and advantages of the invention will be more readily apparent through the following detailed description of preferred embodiments thereof, with reference to the appended drawings, wherein:

Fig. 1 shows the effect of the application of an NT-signal to a rabbit papillary muscle in the presence of Caffeine;

Fig. 2 shows the effect of the application of an NT-signal to a rabbit papillary muscle in the presence of Ryanodine;

Fig. 3 shows the effect of the application of an NT-signal to a rabbit papillary muscle in the presence of Nifedipine;

Fig. 4 shows the effect of the application of an NT-signal to a rabbit papillary muscle in the presence of both propranolol and adrenaline;

Fig. 5 shows the effect of the application of an NT-signal to a rabbit papillary muscle in the presence of adrenaline;

Fig. 6 shows the effect of the application of an NT-signal to a rabbit papillary muscle in the presence of Diltiazem;

Fig. 7 shows the effect of the application of an NT-signal to a rabbit papillary muscle in the presence of Digoxin; and

Fig. 8 is a schematic representation of an apparatus according to one embodiment of the invention.

### Definitions

The following terms and abbreviations, used throughout this specification, are defined below, for the sake of clarity:

b.p.m. = Beats per minute

HMC = Hypertrophic Cardiomyopathy

I.M. = Intramuscular

IV = Intra Venous

LV = Left Ventricle

LVP = left ventricular pressure

NT Signal= Non-Excitatory Signal

RV = Right Ventricle

VF = Ventricular Fibrillation

### **Detailed Description of Preferred Embodiments**

The invention will now be illustrated through detailed experiments. Experiments *in vitro* were carried out using isolated rabbit papillary muscle, and the protocol for its isolation is detailed below.

### **Equipment**

The following equipment which will be referred to hereinafter, is now briefly described for the sake of clarity:

**Plugsys system:** The plugsys system is an incorporating plug in modules for measuring, controlling and data processing in connection with recorders and computers. In general, it functions as an amplifier which increases the sensitivity of the measuring of biological signals. One such device, used in the experiments described herein, is manufactured by HSE, Germany.

**Millar:** This device (manufactured by Millar Instruments, USA), is a micro manometers transducer that can be connected to a battery operated bridge (which is the interface box) and the output can be digitized using an A/D converter. In another mode of operation the transducer is connected through a DBA (plugsys DC Bridge Amplifier), which is an amplifier connected to transducers to measure pressure force (manufactured by

HSE, Germany).

### Isolated Papillary Muscle Protocol

**Animals:** New Zealand white rabbits (males) from Israel (Yokneam) or an hybrid of New Zealand White and local albino rabbits (males, AniLab, Rehovot) are kept in room temperature, 2-3 per cage (35x55x65 cm), under natural light conditions. Daily feeding of dry food (Rabbit Mix- Code 590), and unlimited water supply. The cages and the room are cleaned daily,

### Instruments:

**A. for solution making:** Scales (by Mettler toledo, model P8303, Max 310gram, d=1mGram) **magnetic stirrer.** by Freed electric. Weights 10Kg (d=50gram) by Moznei Shekel, Gas tanks with mixed 95% O<sub>2</sub> +5% CO<sub>2</sub>" pressure regulators, pH meter by Mettler toledo, model 320 PH, ice machine 45 Labotal.

### **B. for the in-vitro papillary muscle preparation**

Dissection chamber (HSH, Hugo Sachs Elektronik, Germany), Steered organ bath type 813 (I-18E) including temperature controller type 319, Force Transducer type F30 with amplifier type 660 and calibration unit (HSE), Stereoscope (Olympus), Digital micro manipulator (HSB), Manipulator, Anti- vibration table (TMC, USA), Faraday cage, Fiber optic illuminator (HSE), Current and Voltage clamp amplifier (axon

Instruments, USA), stimulators (Grass instruments, USA), Micro- pipette puller by Narishige (model pp-83, Japan) Current source ISO 10 and ISO - 50 (home made) supplying 10 and 50mA correspondingly and Oscilloscope, 20MHz (Gould, England), Computers: PowerPC 9500/I50, (Apple, USA), or Pentium, 160MHz, Data Acquisition Boards: PC-AO- 2DC, 16 bite, or the PCI-M10 8, 12 bite board by National Instrument, software: LabView for windows, by National Instrument (USA). Data acquisition and analysis program are home made, The program includes data acquisition and on-line analysis, programmable experiment execution, programmable signal output. The off-line analysis program analyze different parameters of muscle twitch and action potentials.

**Solution:**

The Krebs-Heseleit Solution (KHS) was prepared using materials from Sigma (Israel): 0.32 g/lit KCl (4.5 mM), 6.99 g/lit NaCl (118.0 mM), 2.01 g/lit NaHCO<sub>3</sub> (24.0 mM), 0.285 g/lit MgSO<sub>4</sub>•7H<sub>2</sub>O (1.19 mM), 0.16 g/lit KH<sub>2</sub>PO<sub>4</sub> (1.18 mM), 2.0 g/lit Glucose (11.0 mM), and 0.37 g/lit CaCl<sub>2</sub>•2H<sub>2</sub>O (2.52 mM), added after bubbling with a 95% O<sub>2</sub> + 5% CO<sub>2</sub> gas mixture for 20 minutes.

Solution preparation: Distilled water (ion exchange column Zilion, Israel and ultra filtration by EasypurLF, Israel) are used to prepare the KHS stock solution (X 20, 5L). The chemicals listed above except CaCl<sub>2</sub> are

used. The stock solution expire after 1 week of refrigeration, For each day of experiment fresh solution is prepared (5L) out of the stock solution,  $\text{CaCl}_2$  is added, and the solution is bubbled (95%  $\text{O}_2$ /5%  $\text{CO}_2$ ) for 20 min. and titrated to a pH of 7.4. Bubbled KHS at room temperature is used for perfusion of the papillary muscle kept in the Steiert organ chamber. Ice cold (4 C), bubbled KHS is used for the dissection of the papillary muscles.

**Anesthesia and heart dissection:** animal is brought from the cage to a scale for measuring body weight, The animal is anesthetized by 1Vembutal 1-1.2 mg /Kg body weight I.P, using -5cc syringe and 23 Gage needle. The level of anesthesia is checked by the animal reflex to a pinch. When the animal is deeply anesthetized, the skin over the chest is cut off and the chest wall is cut exposing the heart. Using seizures and a forceps the pericardium is cut and the heart is dissected out by cutting all the blood vessels, Immediately after cutting, the heart is placed in an ice cold and oxygenated KHS.

**Papillary muscle dissection:** The heart is transferred to a fresh ice-cold KHS and than to the dissection chamber, containing ice-cold continuously oxygenated KHS. The heart is fixed to a rubber pad with insect pins and than the left ventricle is opened exposing the papillary muscles. A silk (6 0) thread is tight around the tendon of the papillary muscle and the muscle is dissected out using fine twizers. The dissected muscle (length of 2-3mm) is transferred to the organ bath and the heart is kept in the

dissection chamber at 4°C for further dissections of the other papillary muscles. The dissection of the papillary muscle last for 3-5 min.

**The Steiert Organ Bath:** The muscle is placed in an organ bath, and than fixed to the chamber by a plastic holder. The silk thread tight to the tendon is hooked to a rigid hook on the force transducer (on the opposite side) to give an isometric conditions. The papillary muscle is continuously perfused (7- 12ml/min,) with oxygenated KHS kept at a regulated temperature of 37°C.

**Pacing and Stimulation:**

Pacing stimuli (typically 1Hz, 2ms duration, and amplitude of 2mA) are given by two Ag-AgCl electrodes which are part of the organ bath and are placed under the muscle. The electrodes are covered with AgCl layer, chlorodizing by 5mA, 5ms pulses during perfusion. Constant current stimuli (CCU) are given to the upper part of the muscle using graphite electrodes (diameter of 0.5mm fitted to a glass pipette) placed 2-3mm apart along the fibers' line(contraction axis). The muscle length is adjusted to maximal isometric force and left for equilibration period of 30 min.

**Drug administration:**

We use the perfusion system for drug application by switching the feeding krebs solution bottle to the drug containing bottle. The drug is

administered for a period of 30-80 minutes while monitoring the mechanical and electrophysiological response of the muscle every 10 minutes.

### Example 1

#### Stimulation in the presence of Caffeine

A rabbit papillary muscle was stimulated by an NT-signal in the presence of Caffeine 10mM( Phosfodiesterase Inhibitor ).

A rabbit papillary muscle was stimulated in Organ-Bath perfused by KHS (normal experimental medium) together with Caffeine 10mM (which is a PDE inhibitor and also causes depletion of the internal cardiac  $\text{Ca}^+$  stores - Sigma, Mo, catalog No. C-8960) for 60 min before applying the NT signal.

The NT-signal applied to the electrodes4 produced an average increase in peak force of the control. Caffeine 10mM when applied cause a significant reduction in the basal contraction force (33 % decrease). It also reduced the increase in peak force in response to NT stimulation applied to the same preparat in the presence of Caffeine to an average increase of 15 % in the muscle force, vs 60% increase in the control level.

#### Parameters:

Pacing: 1 Hz, 2mS Duration, 1mA current.

NT signal: 30mS Delay, 60mS Duration, 6mA, with the polarity increasing muscle contractility.

Temp. 36.2°C

The results are shown in Fig. 1.

### Example 2

#### Stimulation in the presence of Ryanodine

A rabbit papillary muscle was stimulated in Organ-Bath perfused by KHS together with Ryanodine (a Ca-release suppresser from the Sarcoplasmic Reticulume). 100nM (Sigma, Mo, catalog No. R-6017) for 60 min before applying the NT signal.

The NT-signal applied to this preparat produced an average increase in peak force of 60% in the control. Ryanodine 100nM when applied cause a significant reduction in the basal contraction force (55% decrease). However when NT stimulation was applied to the same preparat in the presence of Ryanodine, it produced an average increase of 70% in the muscle force, compared to the new basal force.

#### Parameters:

Pacing: 1 Hz, 2mS Duration, 1mA current.

NT Signal: 30mS Delay, 60mS Duration, 6mA.

Temp. 36.2°C

The results are shown in Fig. 2.

### Example 3

#### Stimulation in the presence of Nifedipine

A rabbit papillary muscle was stimulated in Organ-Bath perfused by KHS together with Nifedipine (Ca-channel blocker commonly used in clinical treatment in ischemic heart disease mainly affecting the smooth muscle cells in vascular vessels). 1 $\mu$ M (Sigma. Mo, catalog No. n-7634) for 10 minutes before applying the NT Signal.

The NT signal applied to this preparat produced an average increase in peak force of 30% in the control. Nifedipine 1 $\mu$ M when applied cause a significant reduction in the basal contraction force (70% decrease). However when NT stimulation was applied to the same preparat in the presence of Nifedipine an average of 35% increase in the muscle force was observed.

#### Parameters:

Pacing: 1 Hz, 2mS Duration, 1mA current.

NT Signal: 30 mS Delay, 60 mS Duration, 6mA.

Temp. 36.2°C

The results are shown in Fig. 3.

#### Example 4

##### Stimulation in the presence of Propranolol and Adrenaline

An experiment combining two drugs was carried out, the results of which are shown in Fig. 4. A rabbit papillary muscle was exposed for 5 minutes to propranolol (0.5  $\mu$ M) and adrenaline (0.25  $\mu$ M) in control (low amplitude trace) and activated by the NT signal (high amplitude trace).

The graph depicts twitches of papillary muscle after 5 min exposure to the above. The concomitant presence of the drugs decreased the overall muscle force. When the NT signal was applied, the contractile force increased by about 200%. The control trace reflects the clinical state of heart failure, with reduced contractility, high plasma catecholamine concentration, while there is down regulation of the Beta receptors.

##### Parameters:

pacing: 1Hz, 2 mS duration, 3 mA.

NT Signal: 30 mS delay, 60 mS duration, 6 mA.

**Example 5****Stimulation in the presence of Adrenaline**

An experiment was carried out on a rabbit papillary muscle, in the presence of adrenaline, and the results are shown in Fig. 5. This graph is composed from two different trials on the same papillary muscle (left, rabbit). In the control turning on the NT signal caused an increase of about 110%, then adrenaline, 0.5 $\mu$ M was added to the perfusion chamber. The recording shown in Fig. 5 (on the right) was taken after 5 minutes perfusion with adrenaline. Adrenaline caused a 20% increase in the basal contraction force of the muscle. When the NT signal was turned on there was an additional 86% increase in the peak contraction force. This result indicates that high adrenaline concentration does not attenuate the increase in muscle contraction after NT signal stimulation.

**Parameters:**

Pacing: 1Hz, 2mS duration, 3mA.

NT signal was: 30mS delay, 60mS duration, 6mA.

**Example 6****Stimulation in the presence of Diltiazem**

An experiment was carried out on a rabbit papillary muscle, in the presence of Diltiazem (a Ca-channel blocker commonly used in clinical treatment in ischemic heart disease), and the results are shown in Fig. 6.

A rabbit papillary muscle was stimulated in Organ-Bath perfused by KRS and 10  $\mu$ M Diltiazem, (Lot 702663). The NT-signal applied to this preparat produced an average increase in peak force of 20% under these experimental conditions. The drug caused a significant reduction in the basal contraction force of the muscle.

**Parameters:**

Pacing: 1 Hz, 2mS Duration, 1mA.

NT Signal: 50mS Delay, 50mS Duration, 4mA.

Temp. 36.2°C

**Example 7****Stimulation in the presence of Digoxin**

An experiment was carried out on a rabbit papillary muscle, in the presence of Digoxin, and the results are shown in Fig. 7.

There is a 115% increase in the NT-signal induced force on the control without the addition of the drug. Addition of Digoxin caused an increase of

38% of the basal contraction force. Applying the NT-signal on the Digoxin-treated muscle caused a further increase of 86% on the new basal level, after 19 minutes treatment with Digoxin. The combined Digoxin-NT signal treatment is synergistic, and the increase in muscle contraction, as compared to the single contributions of each treatment, is 20%, as shown in the graph.

Pacing: 1Hz, 2 mS, 3 mA.

NT Signal: 1 Hz, delay 30 mS, duration 60 mS.

Referring now to Fig. 8, a schematic representation of an apparatus according to one embodiment of the invention is seen. In this scheme, a portion of a cardiac muscle, H, is brought into closed positioned relationship with two electrodes, E1 and E2, the ends of which are positioned at root position R1 and R2, respectively. The electrodes receive the voltage and current from a signal generator S, the construction of which is conventional and well known to skilled persons, and which is therefore not described here in detail, which in turn receives power from a power line, PL, connected to an autonomous power source or to the mains, as the case may be. The activity of the power signal generator S is controlled by a controller, C, which may be a microprocessor, or which may be an external controlling device, e.g., a PC or the like computer. The controller C controls the parameters of the signal generated by the signal generator, such as current intensity, frequency and timing, and may use

both preset parameters (e.g., the frequency of pulse generation) and feed-back input, e.g., from apparatus which monitors heart or other parameters, or from a pace maker which supplies the pacing signal. These input signals are collectively schematically indicated in the figure as FB. Of course, the apparatus is only schematically shown, for the sake of brevity. And the skilled person will easily be able to devise many different kinds of apparatus suitable to supply the signal needed in carrying out the invention.

All the above description and examples have been given for the purpose of illustration and are not intended to limit the invention in any way. Many modifications can be carried out in the invention: for instance, many different drugs can be used in conjunction with an NT-signal, or different combinations of drugs can be used; furthermore, different signals can be employed, all without exceeding the scope of the invention.

**Claims**

1. Apparatus for the combined drug/electric stimulation treatment of a cardiac muscle, comprising circuitry for creating a non-excitatory electric potential between at least two points located in the vicinity of a muscle.
2. Apparatus as claimed in claim 1, comprising circuitry for controlling the start time of the electric potential generated between said at least two points.
3. Apparatus as claimed in claim 1, comprising circuitry for controlling the duration of the electric potential generated between said at least two points.
4. Apparatus as claimed in claim 1, comprising circuitry for controlling the magnitude of the electric potential generated between said at least two points.
5. Apparatus as claimed in claim 1, wherein the circuitry for creating a non-excitatory electric potential between said at least two points comprises one or more electrode.
6. Apparatus as claimed in claim 5, wherein the electrodes are carbon electrodes.

7. Apparatus as claimed in claim 1, which is an insertable device.
8. Apparatus as claimed in claim 1, which is an extra corporal device.
9. Apparatus as claimed in claim 1, which is an implantable device.
10. Apparatus as claimed in claim 2 or 3, wherein the circuitry for controlling the start time and/or duration of the electric potential is synchronized to heart activity.
11. Apparatus as claimed in claim 10, wherein the circuitry for controlling the start time and/or duration of the electric potential operate not at every beat of the heart.
12. Apparatus as claimed in claim 11, wherein the circuitry for controlling the start time and/or duration of the electric potential operates every 1, 2 or 3 beats of the heart.
13. Apparatus for the combined drug/electric stimulation treatment, comprising circuitry for causing a non-excitatory electric current to flow between at least two points located in the vicinity of a muscle.

14. Apparatus as claimed in claim 13, comprising circuitry for controlling the start time of the electric current flowing between said at least two points.

15. Apparatus as claimed in claim 13, comprising circuitry for controlling the duration of the electric current flowing between said at least two points.

16. Apparatus as claimed in claim 13, comprising circuitry for controlling the magnitude of the electric current flowing between said at least two points.

17. Apparatus as claimed in claim 13, wherein the circuitry for causing a non-excitatory electric current to flow between said at least two points comprises one or more electrode.

18. Apparatus as claimed in claim 17, wherein the electrodes are carbon electrodes.

19. Apparatus as claimed in claim 13, which is an insertable device.

20. Apparatus as claimed in claim 13, which is an extra corporal device.

21. Apparatus as claimed in claim 13, which is an implantable device.
22. Apparatus as claimed in claim 14 or 15, wherein the circuitry for controlling the start time and/or duration of the electric current is synchronized to heart activity.
23. Apparatus as claimed in claim 22, wherein the circuitry for controlling the start time and/or duration of the electric current operate not at every beat of the heart.
24. Apparatus as claimed in claim 23, wherein the circuitry for controlling the start time and/or duration of the electric current operates every 1, 2 or 3 beats of the heart.
25. Apparatus as claimed in any one of claims 1 to 24, wherein the non-excitatory electric current is a DC electric current.
26. Apparatus for the combined drug/electric-stimulation treatment of a cardiac muscle, comprising:
  - means for creating an electric potential between at least two points located in the vicinity of the cardiac muscle;
  - means for causing a non-excitatory electric current to flow between said at least two point; and

- means for controlling the start time, duration and magnitude of the electric current flowing between said at least two points.

27. Apparatus according to claim 26, comprising:

- means for creating an electric potential between at least a pair of electrodes in the vicinity of the cardiac muscle at at least two root locations;

- means for causing a non-excitatory electric current to flow between said at least two root locations; and

- means for controlling the start time, duration and magnitude of the electric current flowing between said at least two root locations.

28. Apparatus according to claim 26 or 27, further comprising means for superimposing on the DC signal one or more waveforms of given frequency and amplitude, thereby to generate a complex signal.

29. A method for the combined drug/electric stimulation treatment of a cardiac muscle, comprising a) administering to a patient in need thereof a drug which affects the cardiovascular system and which affects the cardiac muscle, or a mixture of two or more such drugs, b) creating a non-excitatory electric potential between at least two points located in the vicinity of the muscle, and c) controlling one or more of the parameters consisting of start time, duration, magnitude and polarity of the non-excitatory electric potential created between said at least two points.

30. A method for the combined drug/electric stimulation treatment of a cardiac muscle, comprising a) administering to a patient in need thereof a drug which affects the cardiovascular system and which affects the cardiac muscle, or a mixture of two or more such drugs, b) causing a non-excitatory electric current to flow between at least two points located in the vicinity of the muscle, and c) controlling one or more of the parameters consisting of start time, duration, magnitude and polarity of the non-excitatory electric current flowing between said at least two points.

31. A method according to claim 30, wherein the non-excitatory electric current is a DC current.

32. A method according to claim 31, further comprising generating a complex signal by superimposing on the DC signal one or more waveforms of given frequency and amplitude.

33. A method according to any one of claims 30 to 32, wherein the flow of the non-excitatory DC electric current is synchronized to heart activity.

34. A method according to claim 33, wherein the non-excitatory DC electric current flows not at every beat of the heart.

35. A method according to claim 34, wherein the non-excitatory DC electric current flows every 1, 2 or 3 beats of the heart.

36. A method for the combined drug/electric-stimulation treatment of a cardiac muscle, comprising:

- administering to a patient in need thereof a drug which affects the cardiovascular system and which affects the cardiac muscle, or a mixture of two or more such drugs;

- providing means for creating an electric potential between at least two points located in the vicinity of the cardiac muscle;

- providing means for causing a non-excitatory electric current to flow between said at least two point; and

- controlling the start time, duration and magnitude of the electric current flowing between said at least two points so as to impart a desired change in cardiac muscle contractility.

37. A method according to any one of claims 29 to 36, wherein the change in cardiac muscle contractility is designed at least partially to compensate for cardiac muscle contractility decrease induced by said drug or mixture of drugs.

38. A method according to any one of claims 29 to 36, wherein the change in cardiac muscle contractility is designed at least partially to compensate

for cardiac muscle contractility increase induced by said drug or mixture of drugs.

39. A method according to any one of claims 29 to 36, wherein the change in cardiac muscle contractility is designed to add to and/or amplify the cardiac muscle contractility increase or decrease induced by said drug or mixture of drugs.

40. A method according to any one of claims 29 to 36, for reducing the dosage of a cardiovascular drug which affects cardiac muscle contractility.

41. A method according to any one of claims 29 to 36, for reducing the dosage of a cardiovascular drug which affects the electrophysiological characteristics of the cardiac muscle.

42. A method for the combined drug/electric-stimulation treatment of a cardiac muscle, comprising:

- administering to a patient in need thereof a drug which affects the cardiovascular system and which affects the cardiac muscle, or a mixture of two or more such drugs;
- providing an electric potential between at least a pair of electrodes in the vicinity of the cardiac muscle at at least two root locations;
- causing a non-excitatory electric current to flow between said at least two contacting locations; and

- controlling the start time, duration and magnitude of the electric current flowing between said at least two root locations, so as to impart the desired change in cardiac muscle contractility.

43. A method according to claim 42, wherein the means for causing a non-excitatory electric current to flow, are synchronized to heart activity.

44. A method according to claim 43, wherein the means for causing a non-excitatory electric current to flow operate not at every beat of the heart.

45. A method according to claim 44, wherein the means for causing a non-excitatory electric current to flow operate every 1, 2 or 3 beats of the heart.

46. A kit for the treatment of cardiovascular diseases, comprising a drug or a combination of two or more drugs, and an apparatus according to any one of claims 1 to 29.

47. Apparatus as claimed in any one of claims 1 to 26, comprising circuitry for controlling its activity as a function of calculated and/or actual level of a given drug or combination of drugs in the patient's blood.

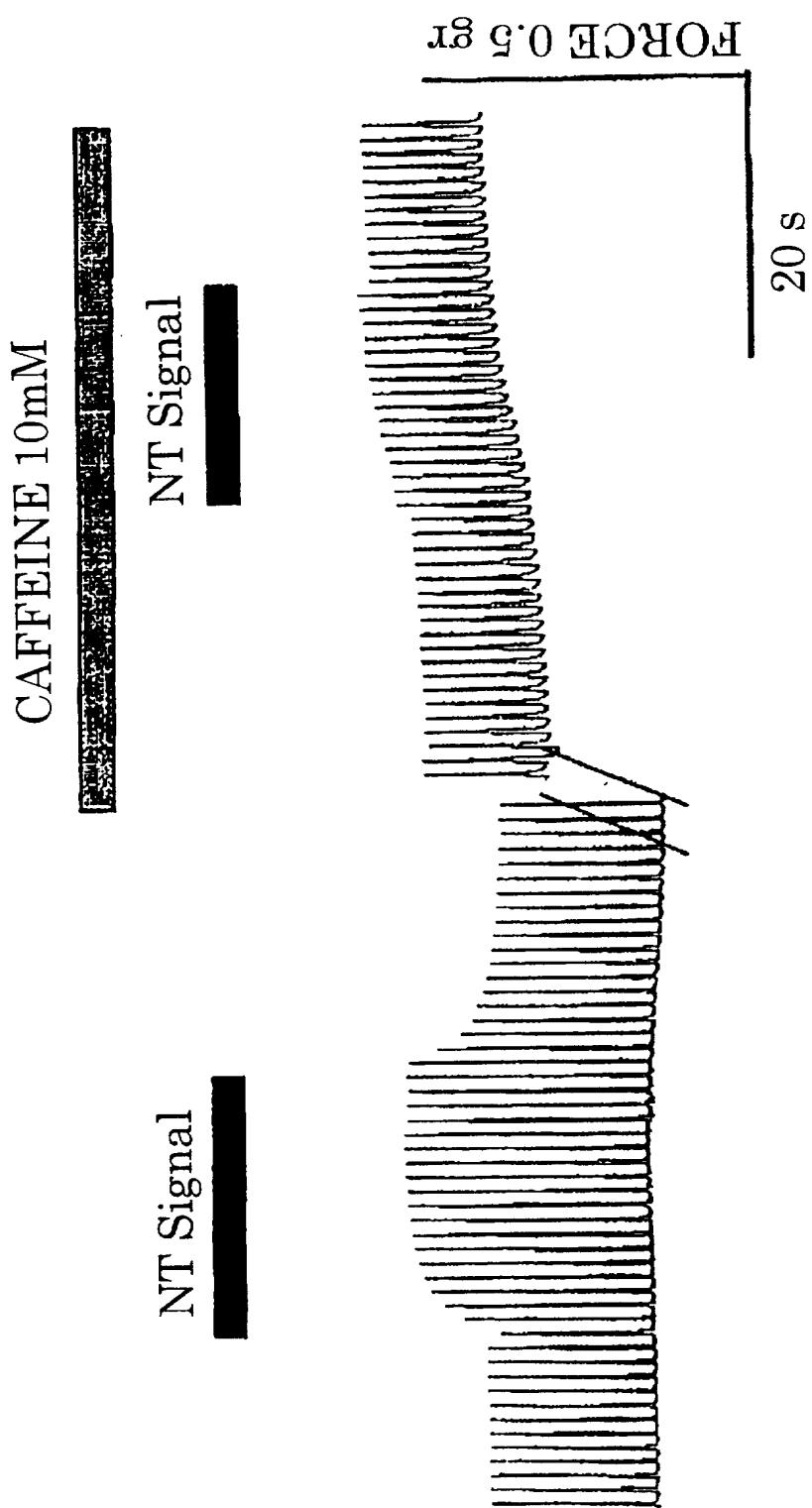
48. Apparatus according to any one of claims 1 to 26, for use in reducing the dosage of pro-arrhythmic drugs.

49. Apparatus according to any one of claims 1 to 26, for use in counteracting side-effects of pro-arrhythmic drugs.

50. Apparatus according to any one of claims 1 to 26, comprising timing circuitry for activating it at predetermined times during the day.

51. A method for the combined drug/electric-stimulation treatment of a cardiac muscle, essentially as described and with particular reference to the examples.

1/8



*Fig. 1*

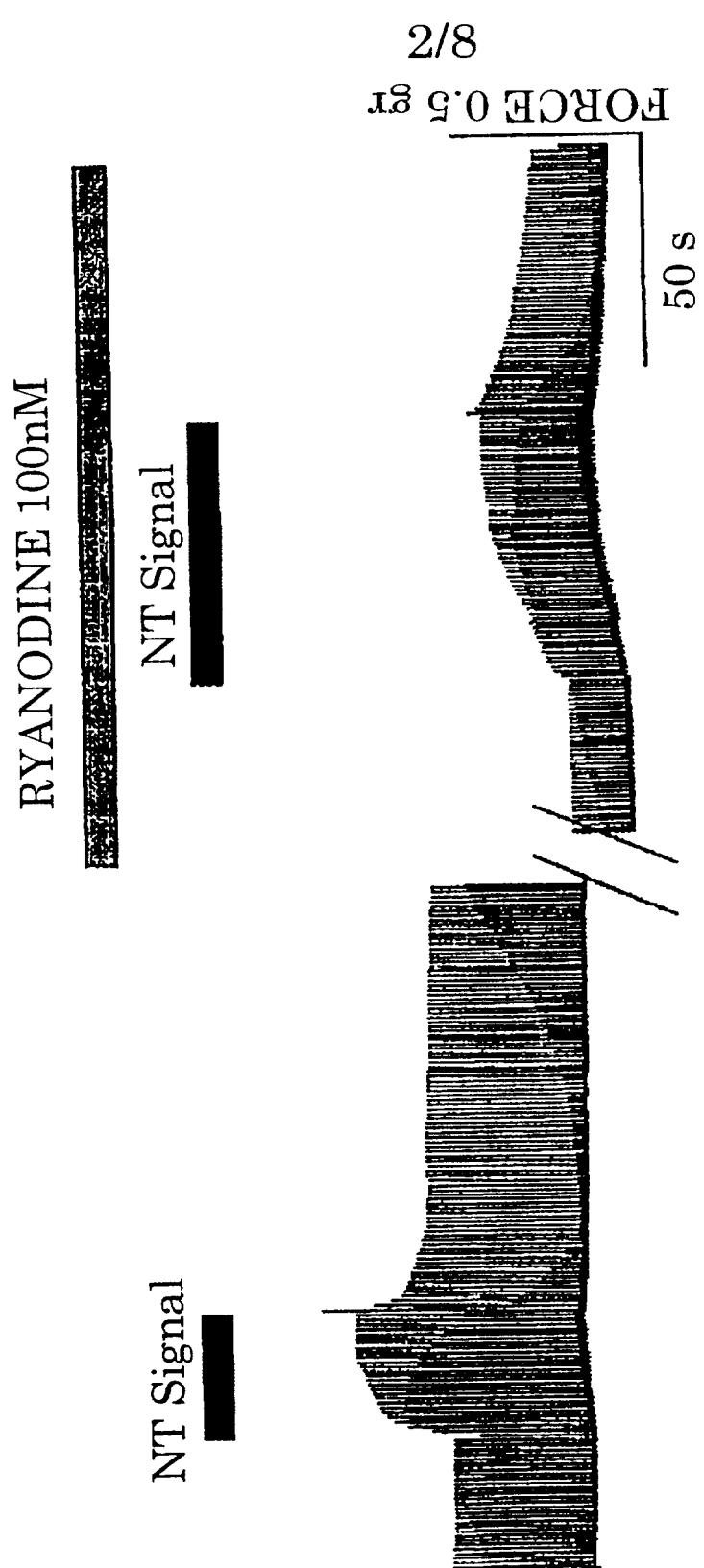


Fig. 2

3/8

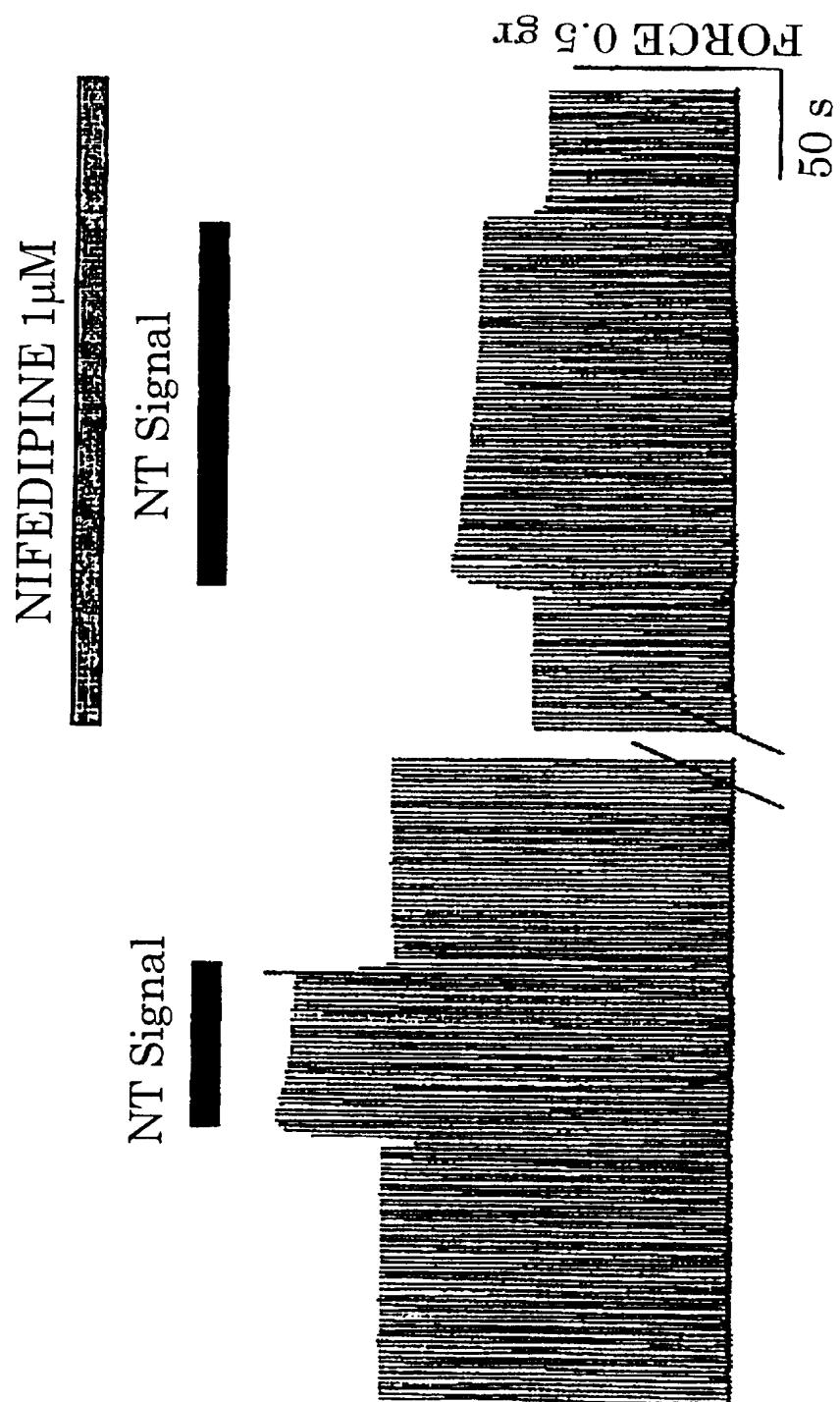
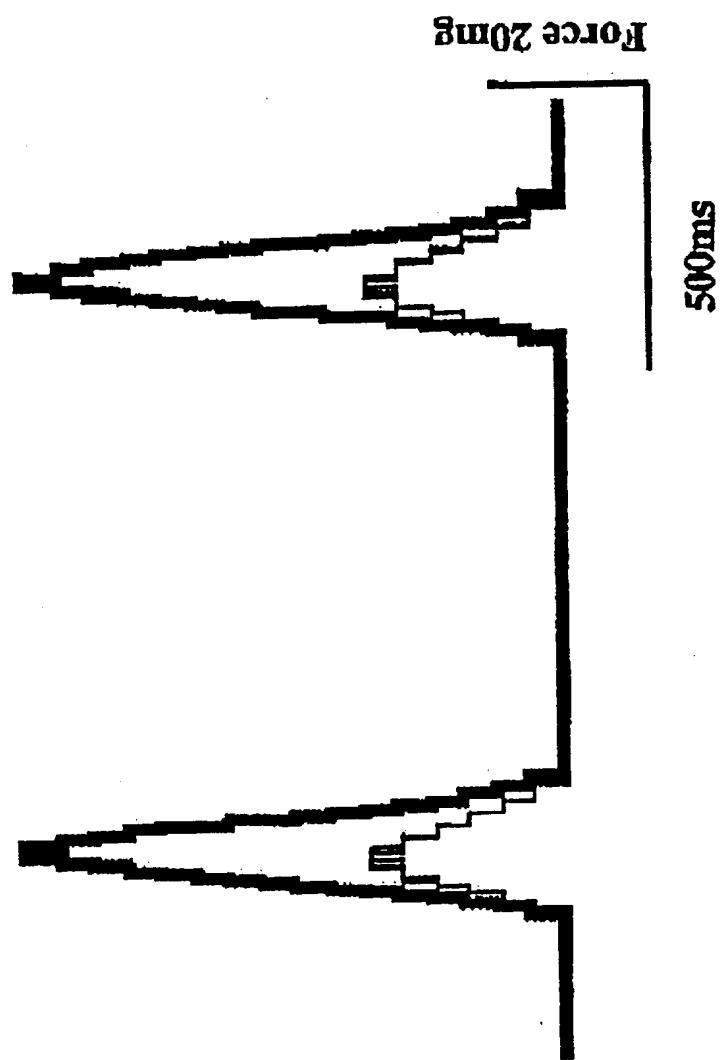
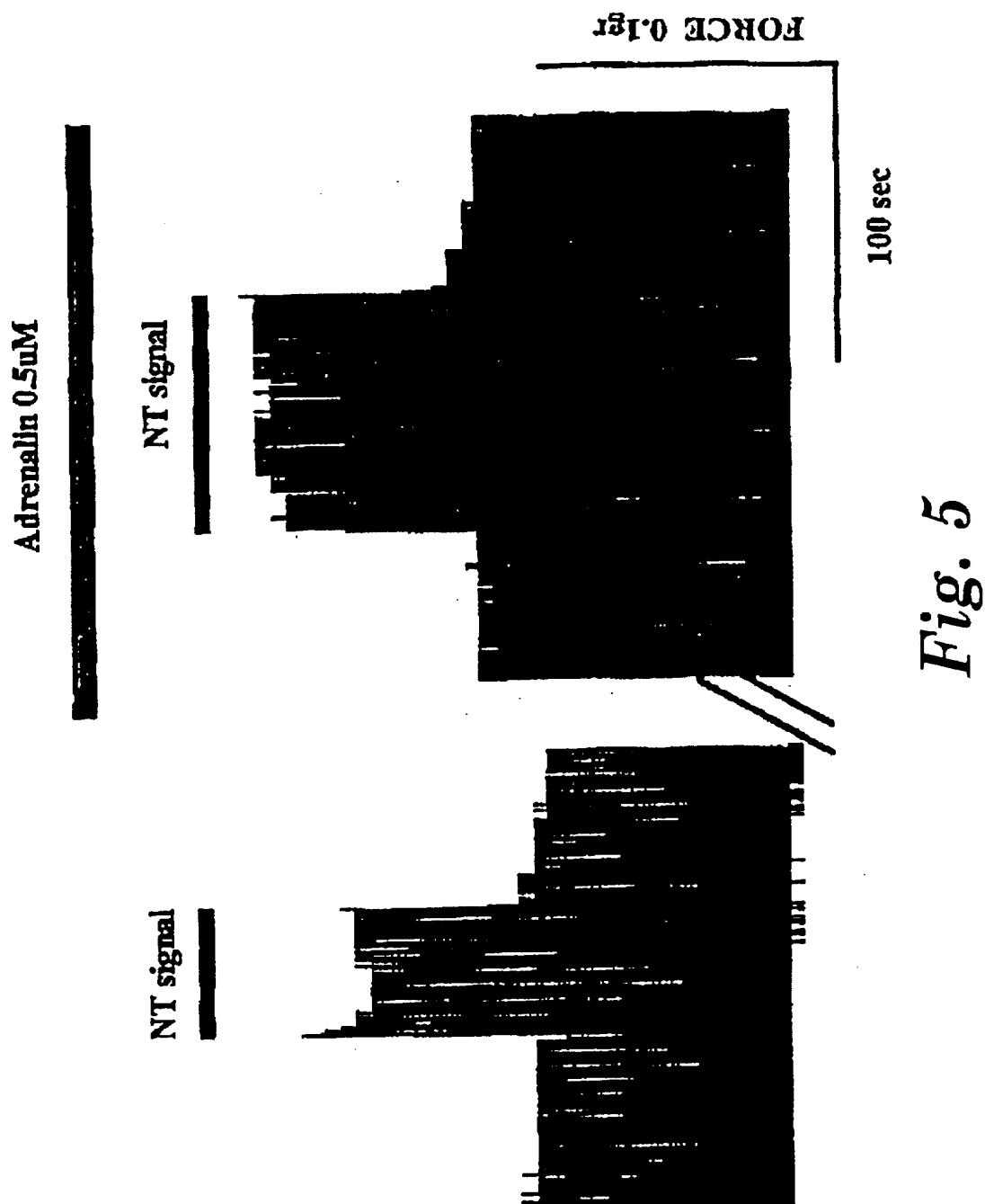


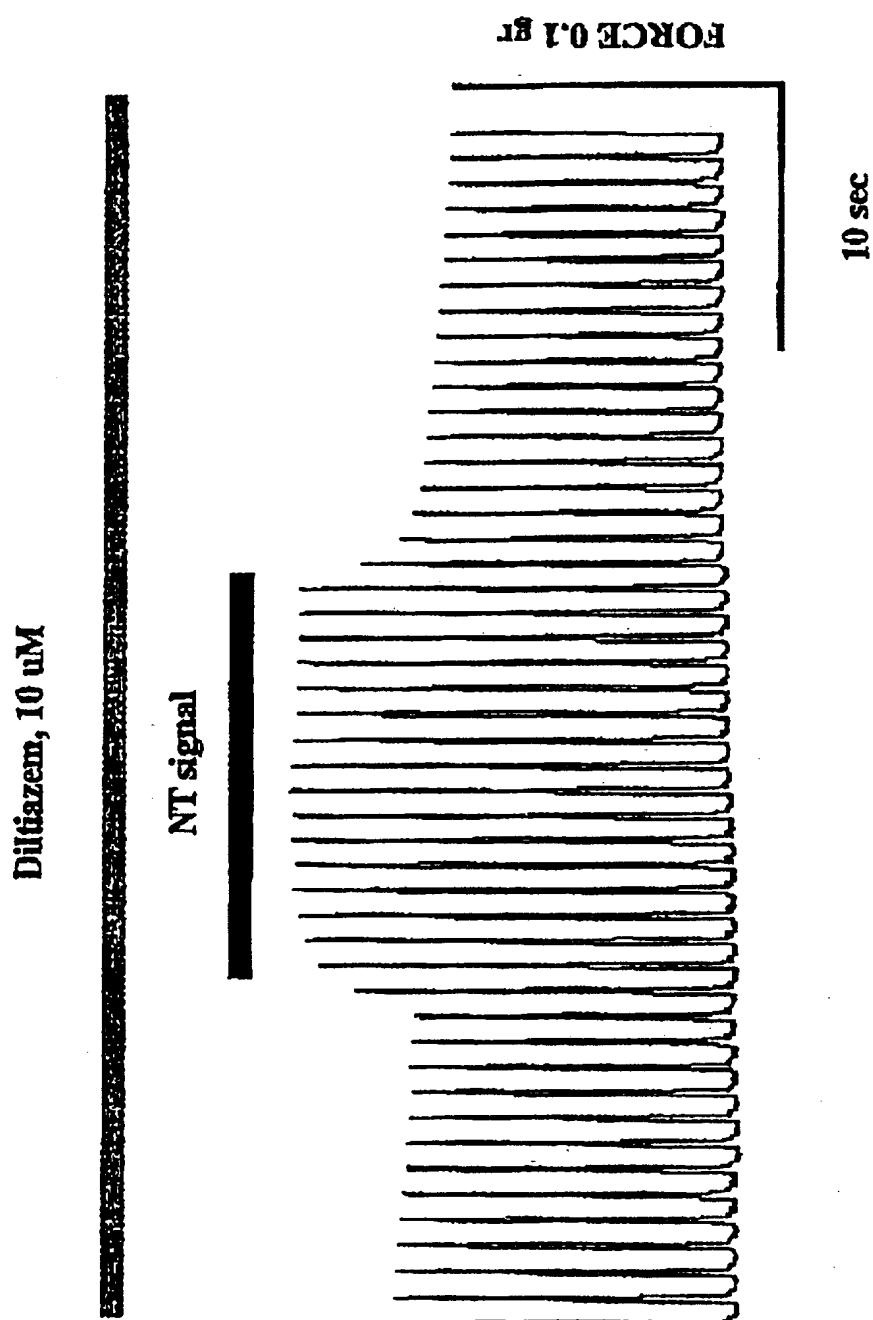
Fig. 3



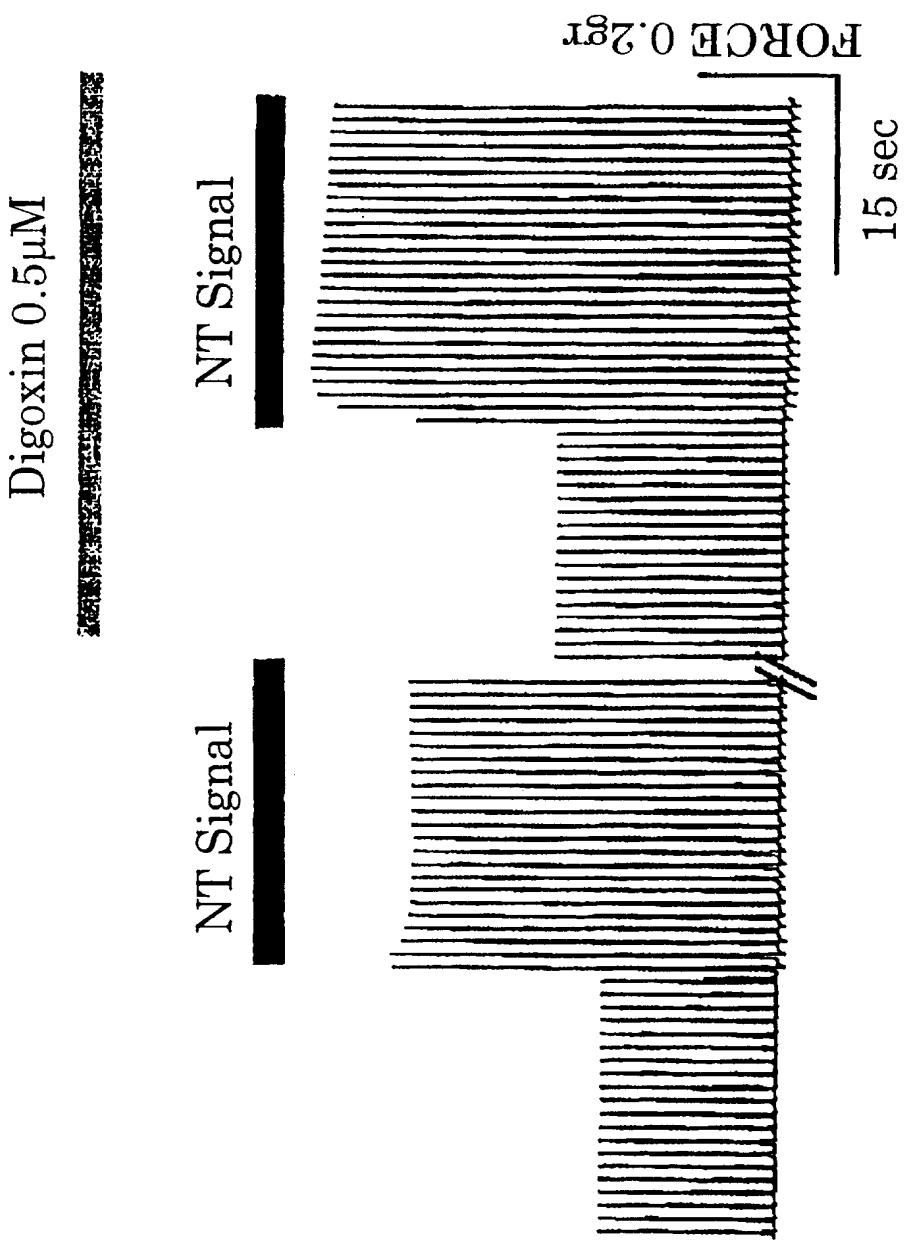
*Fig. 4*

5/8





7/8



*Fig. 7*

8/8

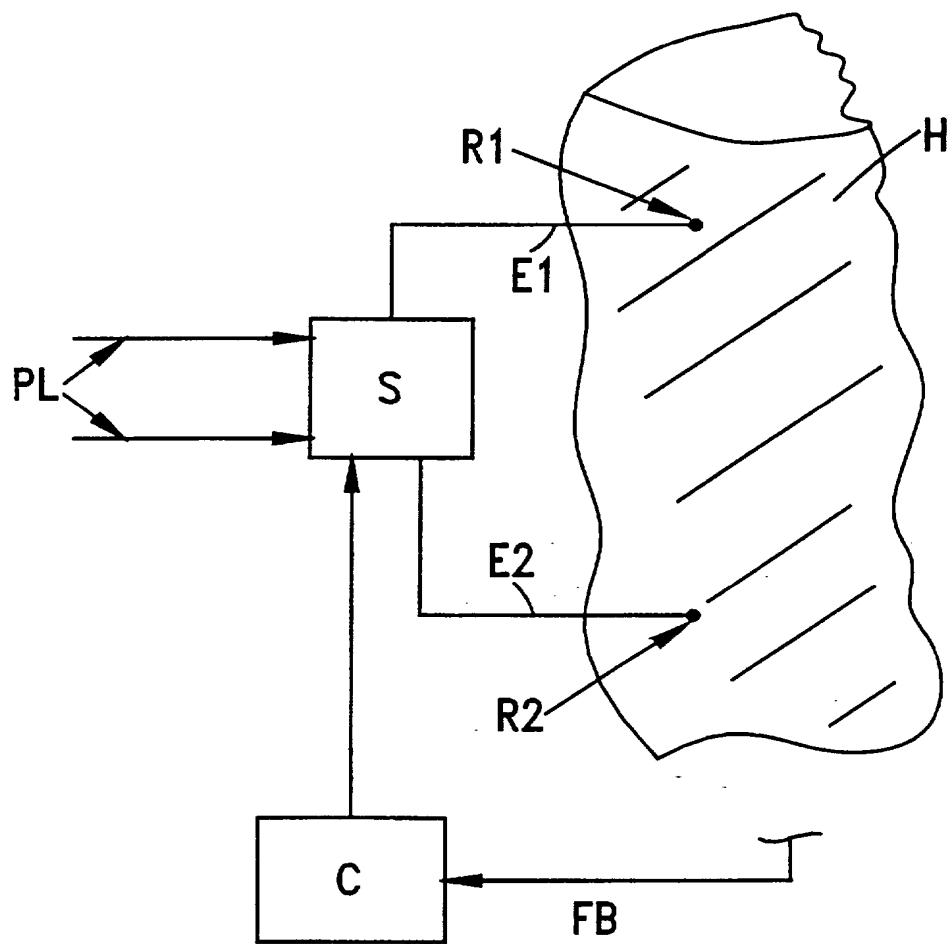


Fig. 8

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/IL97/00232

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61N 1/362

US CL : 607/009

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 607/003, 009, 010, 072

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,083,564 A (SCHERLAG) 28 JANUARY 1992, SEE ENTIRE DOCUMENT.	I-28 & 49-51

Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y"	document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"g."	document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means		
"P" document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

Date of mailing of the international search report

**13 OCT 1997**

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231  
Facsimile No. (703) 305-3230

Authorized officer  
JEFFREY R. JASTRZAB  
Telephone No. (703) 308-0858

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/IL97/00232

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.: 29-45 and 52  
because they relate to subject matter not required to be searched by this Authority, namely:  
A search is not required for medical methods per PCT Rule 39.
2.  Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3.  Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1.  As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4.  No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

### Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.